THE RELATIONSHIP BETWEEN DIETARY SODIUM AND CALCIUM METABOLISM IN MEXICAN-AMERICAN ADOLESCENTS

by

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Introduction

US Mexican-American children and adolescents, on average, are consuming 3204 mg per day of sodium and sodium intake is increasing with age (1). The adequate intake value for children 9-18 y is 1500 mg per day of sodium and the upper limit is 2250 mg sodium per day (2). On average all children including Mexican- American children are exceeding the sodium upper limit (1). Increasing dietary sodium intake promotes an increase in urinary calcium excretion (3-5). Dietary sodium intake has been analyzed by measuring urinary calcium excretion in epidemiologic studies. Urinary sodium excretion has been studied in men (6,7), women (6-8), and young females (8-13 y) (9). Adolescence is of particular concern for excessive sodium consumption because almost 40% of adult peak bone mass is developed during those years (10).

Race is also an important determinant of calcium retention. The relationship between sodium intake and calcium retention is also race dependent (11). Black adolescent girls retain more calcium than white girls on the same diet (12). Blacks retained more calcium than whites on both high- and low-sodium diets. There was greater sodium retention (1190 mg) in blacks consuming a high sodium diet (3860 mg/day) compared to whites (375 mg) consuming the same diet, because whites were excreting more sodium in the urine. Sodium-calcium relationships have not yet been studied in Mexican-American adolescents. The hypotheses are that urinary calcium and sodium excretion will reflect varying intakes of dietary calcium and sodium intake in Mexican-American adolescents. Studying the difference in effect of dietary sodium on sodium and calcium retention among races is important to understand racial differences in vulnerability to diseases such as osteoporosis and can influence guidelines for calcium and sodium intakes for specific racial groups.

This study utilized samples from a parent study whose objective was to determine the effect of calcium intake on calcium retention in Mexican-American boys and girls. The parent study used a cross-over design of low and high calcium intake. The hypotheses of this study were tested cross-sectionally as each participant had one sodium intake for both interventions, but a range of sodium intakes (albeit somewhat narrow) was studied due to the range in energy intake of the diet.

Subjects and Methods

Subjects

Healthy teens of Mexican-American decent were recruited from schools in Indiana and Illinois. Twenty-three girls aged 12-14 y and twenty-three boys aged 13-15 y responded to flyers that were distributed by filling out a screening questionnaire to determine if they were qualified for the study. Both parents and grandparents were to be of Mexican-American descent. Exclusion criteria included the following: younger than 11 y or older than 15 y, body mass index (BMI) of less than 15^{th} or greater than 85^{th} percentile for age, history of pregnancy or abortion, eating disorders, oral contraceptive use, tobacco use, malabsorptive disorders, bone, liver or kidney disease, or hormonal abnormalities. Questionnaires and physical examinations were used to determine the health status of subjects. A staff physician verified the health of the individuals with general blood chemistry panels and general physical examination. The Institutional Review Boards of Purdue University and Indiana University School of Medicine approved protocols. Teens and their guardians gave informed consent.

Design

The crossover metabolic balance study consisted of two 3-week sessions separated by a 1-week washout period. Each subject was randomly assigned to one of five paired high/low calcium intakes covering a range of ~600-2300 mg Ca/d (Table 1)

Table 1. Calcium Intake (mg/day) Assignments

Group	Lower Level	Higher Level
1	600	1100
2	800	1300
3	900	1500
4	1000	1900
5	1200	2300

Participants were housed in a campus residence hall at Purdue University for the duration of the study. Daily schedules were designed to include educational and recreational activities. Participants were required to consume all meals, snacks, and beverages that were provided. All urine and feces were collected for the duration of the study (each 3-week metabolic period).

Diet

Participants were asked to complete six, 24-h recalls prior to the study to assess usual diet. Records were analyzed using a nutrient database (Nutrition Data System for Research, 2011, Nutrition Coordinating Center, University of Minnesota). Throughout the study, subjects were assigned a caloric level (1750 kcal/d – 3000 kcal/d) to meet individual needs. The base diet contained 600 mg/d of calcium and other minerals (sodium, phosphorous, etc.) varied depending on the caloric intake of the individual. Sodium intake was dependent on the calorie levels

assigned. The menu was adjusted to meet the caloric levels through adjustment of caloric beverages and foods. Deionized water could be consumed freely. Composition of menu was assessed using composites of each meal. Meal composites were prepared for mineral analysis through a process of homogenization, freeze-drying and ashing. Samples were analyzed in duplicate using inductively coupled plasma spectrophotometry (ICP, Optical Emission Spectrometer, Optima 4300DV;Perkin-Elmer, Shelton, CT). These mineral analysis data was used for balance calculations.

Participants were assigned one of five experimental paired calcium intakes, either high/low or low/high. The dietary calcium intake ranged from ~600-2300 mg/d. During the first 3-week period of the study, subjects consumed one level of calcium intake and consumed a different level during the second 3-week period (Table 1). Orange juice was manipulated to contain necessary experimental calcium above the basal diet. This was accomplished through blending fortified and unfortified orange juice with calcium citrate malate.

Compliance

During mealtimes, subjects were monitored in a controlled, supervised environment. Meals were served in containers specifically coded for their assigned diet. Subjects were not permitted to exchange or discard food. Consumption of all food was encouraged, but foods that were not consumed during the mealtime were logged. Leftover foods were wrapped, stored and offered at a later meal or snack.

Urine was collected for the entirety of each 3-week metabolic period of the study from all subjects. Subjects were given acid washed, labeled containers to collect urine in during each 3-week metabolic period. Counselors were responsible for monitoring bathrooms 24 h/d. If a subject missed a sample or spilled during collection, it was noted on a log. Compliance of urinary collection was assessed by analysis of creatinine daily.

Measurements

Collected urine samples for each subject were pooled every 24 hours, ending with the rising collection at the beginning of each day. Volume of daily samples was measured and acidified with hydrochloric acid (1% by volume). Samples were then frozen for storage. Samples were thawed and diluted with 3% HNO3 and were analyzed for calcium and sodium by inductively coupled plasma spectrophotometry (Optical Emission Spectrometer, Optima 4300DV; Perkin-Elmer, Shelton, CT).

Calcium and Sodium Calculations

Data from the parent study were used for secondary analysis for this honors project. The crossover design was eliminated for analysis and data were used from subjects if they completed the metabolic period (completion of 3-week period). Equilibration to the basal diet was achieved in the first seven days of each 3-week period, and the following fourteen days were used for calculations. Average sodium in the urine was calculated for each subject by taking the mean of urinary sodium from the last fourteen days of each metabolic period. Mean calcium in the urine was calculated using an average of corrected urinary calcium excretion over the last fourteen days of the metabolic period. Corrected urinary calcium is a measure of urine collection

compliance based on daily urinary creatinine excretion and to adjust for incomplete 24-h collections. It is calculated by multiplying average creatinine excretion by urinary calcium excretion on a particular day divided by creatinine excretion on that same day. Calcium retention was calculated by subtracting excretion of calcium (urinary and fecal calcium) from dietary intake of calcium. Percent calcium retention was calculated by dividing calcium retention by mean dietary calcium.

Statistical Analysis

P values were calculated using the GLM procedure. A linear regression model was developed, describing the slope of the trend line. Statistical significance was set at $P < 0.05$. R^2 values were calculated to determine the fraction of variation in y that is described by x. All statistical analyses were done using SAS software (version 9.2; SAS Institute, Cary, NC). Of the 23 boys and 23 girls recruited for the study, 20 girls (34 observations) and 21 (38 observations) boys were used for this analysis. 1 boy and 3 girls left the study within the first six days and 1 boy was eliminated because of poor compliance. Subjects were eliminated if they did not complete the 3-week metabolic period.

Results

Physical characteristics, usual calcium intake and bone mineral density (BMD) are shown in **Table 2**. Boys were taller, had higher lean body mass, usual calcium intake and bone mineral content compared to girls, while girls had higher tanner score and fat mass compared to boys ($P < 0.05$). There were no significant differences in any of the other variables.

Figure 1 shows that the relationship between dietary sodium intake and urinary sodium in Mexican-American adolescents. As dietary sodium intake increased, urinary sodium excretion increased ($P < 0.05$). **Figure 2** shows that the relationship between dietary calcium intake and urinary calcium excretion in Mexican-American adolescents. As dietary calcium increased, urinary calcium excretion also increased ($P < 0.05$). **Figure 3** shows dietary sodium intake as a function of urinary calcium excretion in Mexican-American adolescents. As dietary sodium intake increased, urinary calcium excretion decreased ($P > 0.05$).

When comparing the relationship between dietary sodium intake with percent calcium retention in Mexican-American girls with that measured in previous studies (11) in black and white adolescent girls (**Figure 4**), we found that percent calcium retention as a function of sodium intake in Mexican-American girls falls in between percent calcium retention of white and black girls. Percent calcium retention of Mexican-American boys falls above that of Mexican-American girls, but still is in between percent calcium retention of black and white girls.

Discussion

Calcium retention in adolescent black and white girls is impacted by dietary sodium intake (12). Few studies have investigated the impact of dietary sodium on calcium retention in Mexican-American adolescents to understand how high dietary sodium intake leads to decreased calcium retention in that population. In our study, urinary sodium and urinary calcium excretion were significantly affected by dietary sodium and dietary calcium intake, respectively in Mexican-American adolescents. The relationship between urinary calcium excretion and dietary sodium intake was not significant in Mexican-American adolescents over a small range of sodium intake.

The relationship between dietary sodium intake and urinary sodium excretion in Mexican-American adolescents has a positive slope, as does the same relationship in white and black girls (11). As dietary sodium increases, urinary sodium increases. Using the equation of the trend line, when dietary sodium intake is 3860 mg/d urinary sodium excretion is 3140 mg/d in Mexican-American adolescents. It can be predicted that 81.4 % of dietary sodium is excreted. In black adolescent girls, 69.2 % of dietary sodium is excreted and in white adolescent girls 90.2 % of dietary sodium is excreted (11). It can also be predicted that on a high sodium diet (3860 mg/d) Mexican-American adolescents would have urinary sodium levels between white and black girls. Wigertz et al found that black girls excrete more sodium on a high sodium diet and white girls excrete less, therefore white girls retain more sodium than black girls do (11). On a high sodium diet, black girls will excrete more sodium and white girls will excrete less sodium than Mexican-American adolescents.

In Mexican-American adolescents, as dietary calcium intake increases, urinary calcium excretion increases. These results are comparable to results from previous studies (9,13). Matkovic et al studied this relationship in 370 young females (9). Braun et al studied this relationship in white and black girls (13). The trendline resulting from the current study has a positive slope and is similar to the trendlines produced from previous research. More research needs to be done to examine racial differences between Mexican-American, white, and black girls in regards to this relationship.

The relationship between dietary sodium and urinary calcium excretion in Mexican-American adolescents was not significant. The trendline produced has a negative slope, which is opposite of expected results. Previous research indicates that as dietary sodium increases, urinary calcium increases (9). The current study produced a trendline with a negative slope, suggesting that in this population as dietary sodium intake increases, urinary calcium excretion decreases. These results were insignificant and a wider dietary sodium range would need to be used to understand the true relationship.

Mexican-American boys and girls fall in between black and white girls with regard to the effect of dietary sodium intake on percent calcium retention. Both Mexican-American boys and girls are closer to white girls than black girls. However, Mexican-American girls appear to fall closer to the trendline for white girls than Mexican-American boys do. This is because of gender differences between boys and girls in calcium retention. Boys have higher calcium retention than girls on the same calcium intake. The calcium retention in Mexican-American girls is closer to white than black girls reflecting bone mineral density (BMD), as Mexican-American girls have BMD values closer to whites than blacks (12, 13).

The parent study was designed to test the effect of dietary calcium intake and the effect of dietary sodium was a secondary question. To more effectively establish the effect of dietary sodium intake on calcium and sodium excretion and calcium retention, a study needs to be designed that includes a larger sodium intake range. The study by Wigertz et al used two sodium intake levels which represented the 5^{th} and 9^{th} percentiles of sodium intake for adolescents, i.e.

1300 mg and 3860 mg sodium daily (11). The current study was limited to 2570 mg - 3649 mg sodium daily. This range was a very small range compared to the Wigertz et al study.

Race and diet play an important role in accretion of bone mass during critical adolescent years. Dietary sodium may play an important role in bone development. Racial differences in the metabolism of sodium and calcium exist. Dietary sodium affects bone accretion more in whites than in blacks (11), and may affect Mexican-Americans less than whites but more than blacks. Mexican-Americans are likely quite vulnerable to dietary sodium intake compromising bone mineral density in adolescence. Their high intakes of dietary sodium may partially explain the high risk of fracture reflected by odds ratio for fracture of Mexican-American women (15).

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Table 1. Calcium Intake (mg/day) Assignments

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Table 2. Baseline physical and body characteristics of the subjects (mean±SD)

Characteristics	**Boys (N=21)**	**Girls (N=20)**
Age (yr)	14.2 ± 1.0	13.6 ± 1.0
Height (cm)	170.9 ± 6.9	158.3 ± 5.1 *
Weight (kg)	78.8 ± 25.7	68.0 ± 18.3
Tanner score[2]	2.7 ± 0.9	3.8 ± 0.7 *
Post-menarcheal age (months)	-	18.4 ± 12.5
Body Mass Index (kg/m^2)	26.8 ± 8.0	27.2 ± 7.6
Lean body mass (%)[3]	65.0 ± 10.6	57.1 ± 6.3 *
Fat body mass (%)[3]	31.5 ± 11.3	39.5 ± 6.8 *
Usual Ca intake (mg/d)[4]	904.06 ± 406.31	648.5 ± 191.9*
Bone mineral content (g)	2634.5 ± 425.3	2188.9 ± 276.6*
Bone mineral density (g/cm3)	1.114 ± 0.108	1.094 ± 0.097

* Significantly different from boys using T-test at $p<0.05$

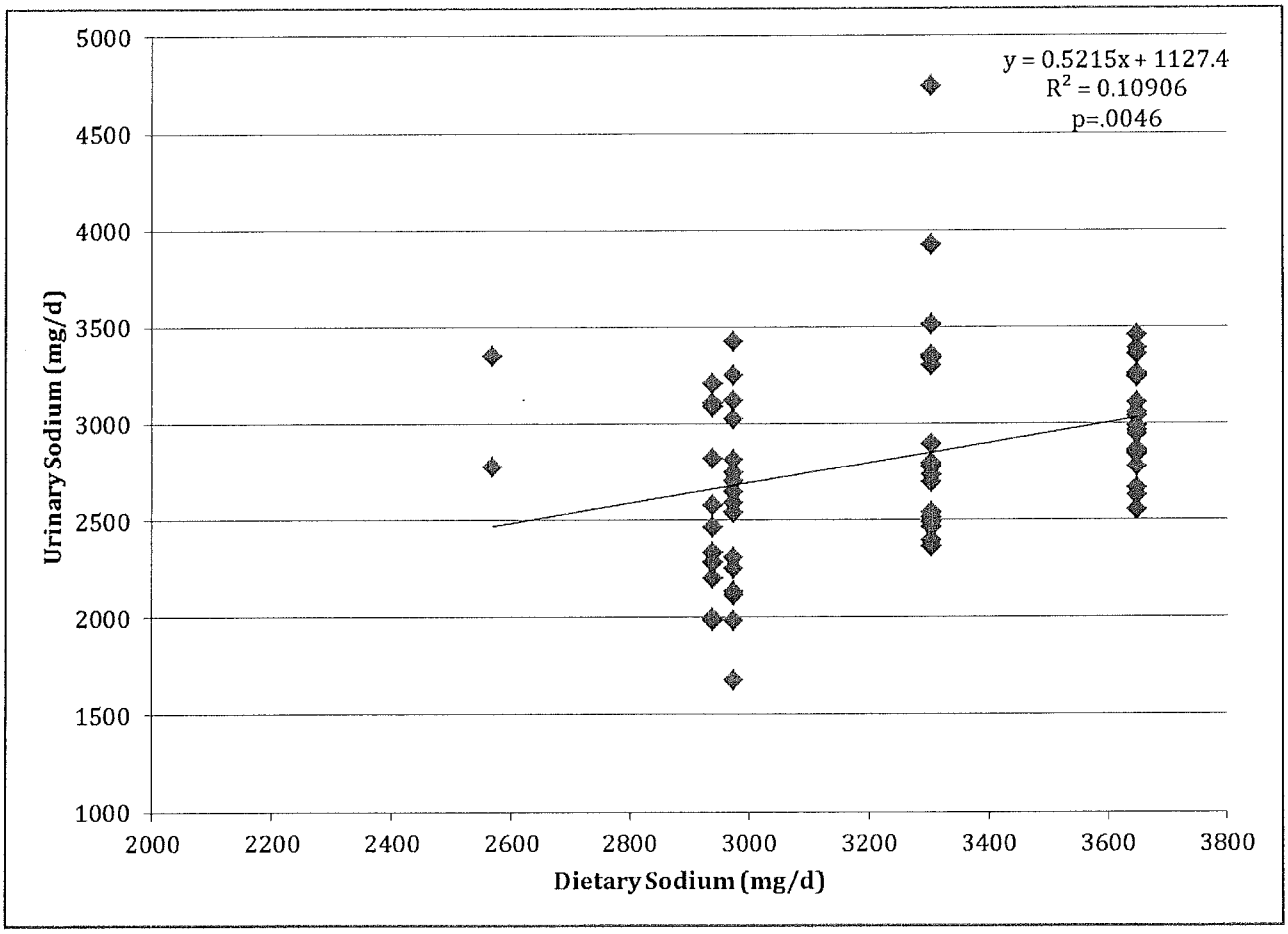

Figure 1. Relation between dietary sodium intake and urinary sodium excretion in Mexican-American adolescents. (n = 41) As dietary sodium intake increases, urinary sodium excretion increases ($P < 0.05$).

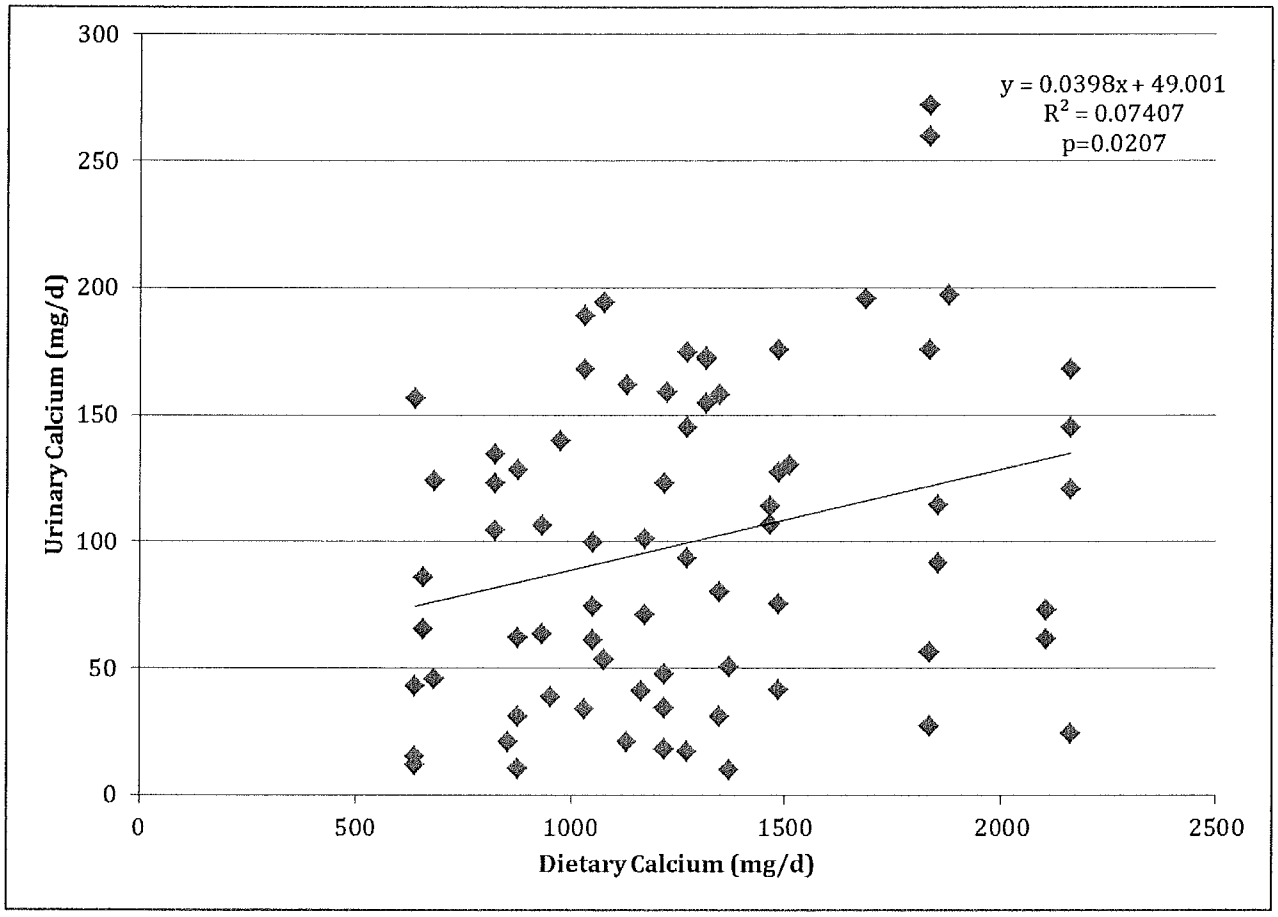

Figure 2. Relation between dietary calcium intake and urinary calcium excretion in Mexican-American adolescents. (n = 41) As dietary calcium intake increases, urinary calcium excretion increases ($P < 0.05$)

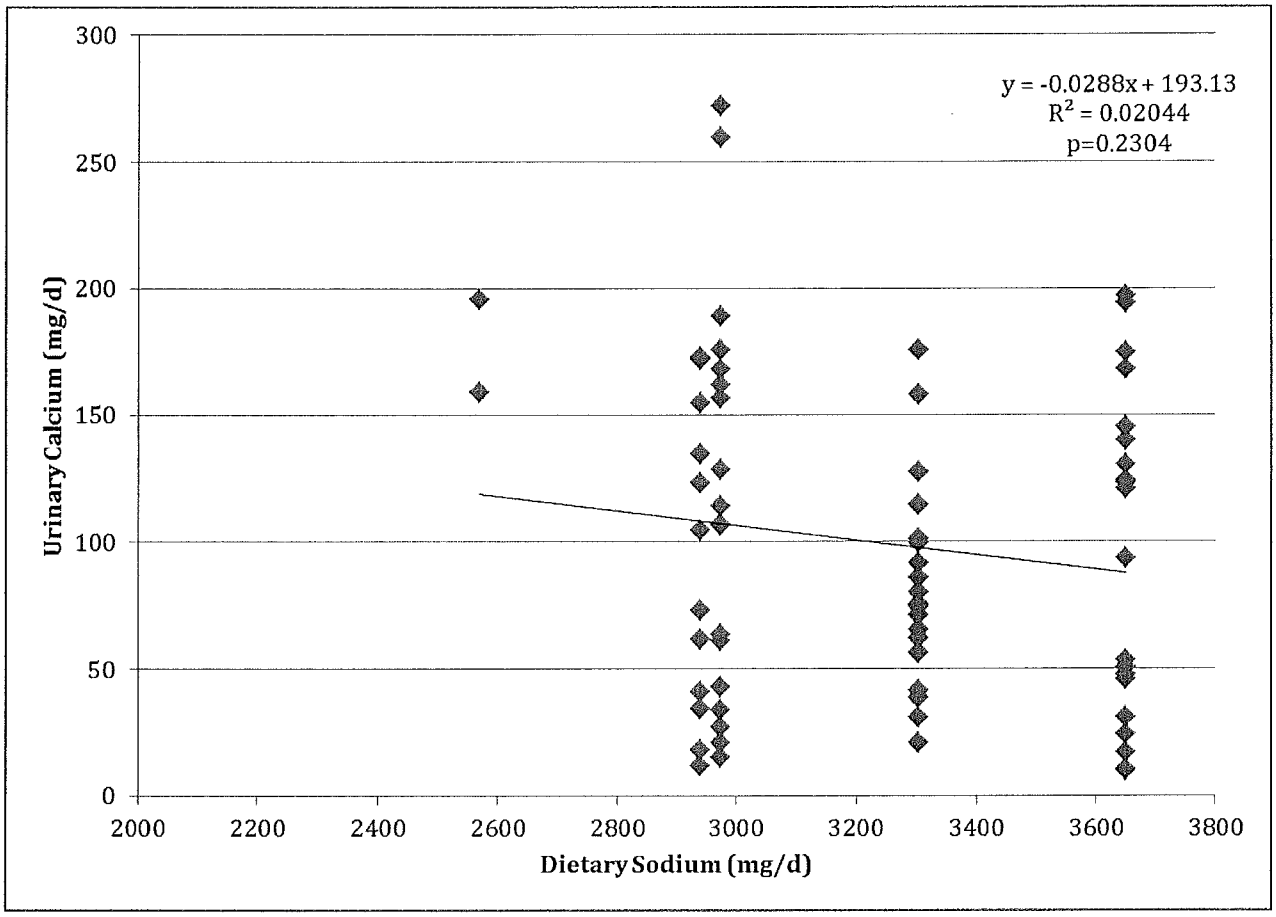

Figure 3. Relation between dietary sodium intake and urinary calcium excretion in Mexican-American adolescents. (n = 41) The relationship between dietary sodium intake and urinary calcium excretion was not statistically significant ($P > 0.05$).

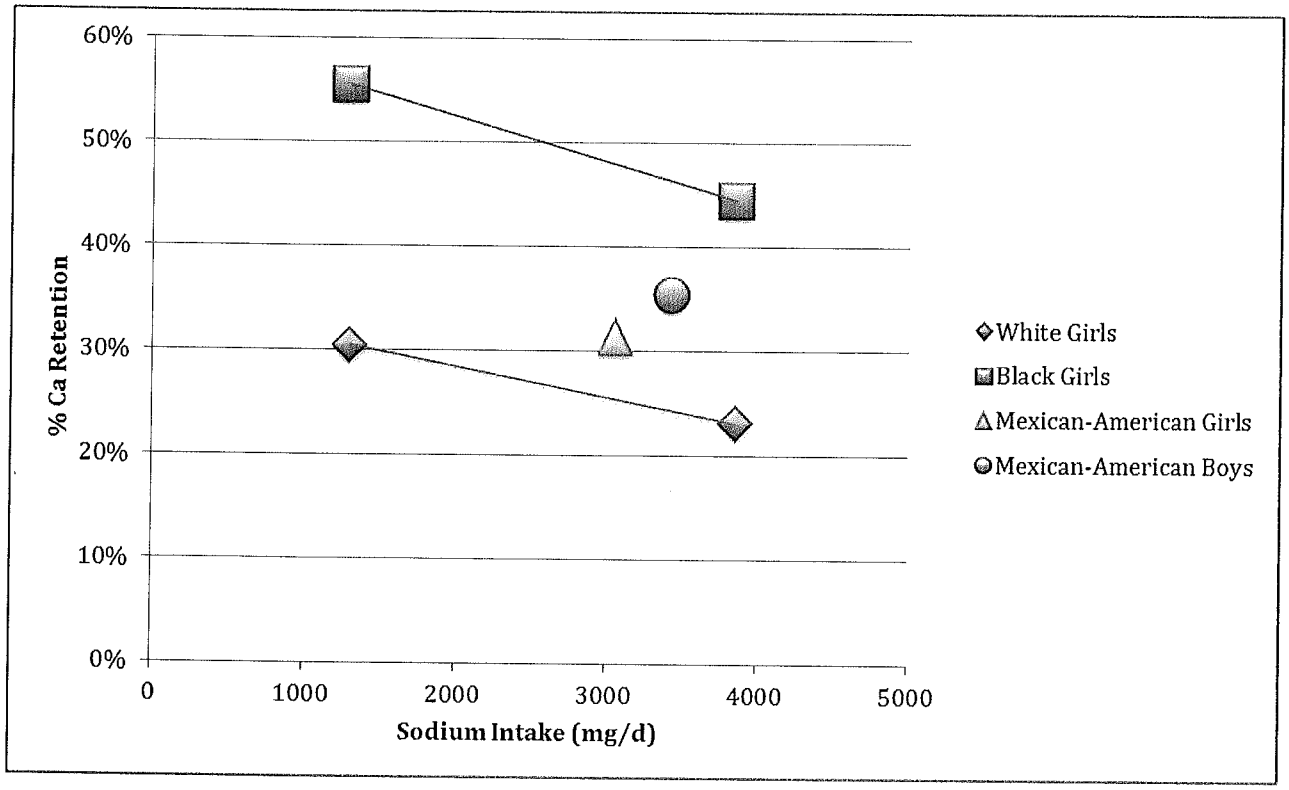

Figure 4. Relation between sodium intake and percent calcium retention in white, black and Mexican-American adolescent girls and boys. Mexican-American girls (n = 20) are more similar to whites than blacks in regards to percent calcium retention as a function of dietary sodium intake (11). Mexican-American boys (n = 21) have higher percent calcium retention than Mexican-American girls.